

Temperature Effects on Growth and Culture Stability of *Chlorella* sp. in Laboratory Cultivation

Pengaruh Suhu terhadap Pertumbuhan dan Stabilitas Kultur *Chlorella* sp. dalam Pengamatan di Laboratorium

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ABSTRACT

The growth behavior of chlorella sp. was evaluated under different temperature conditions to examine how thermal variation influences microalgal biomass production and culture dynamics. Cultivation experiments were conducted at 28, 35, and 40 °C under continuous illumination with aeration. Biomass accumulation was monitored through optical density measurements at 680 nm, while physicochemical parameters including pH and total dissolved solids (TDS) were recorded throughout cultivation. Stable biomass accumulation was observed at 28 °C, whereas growth became less consistent at 35 °C and declined at 40 °C, indicating increasing thermal stress at higher temperatures. Variations in pH and TDS during cultivation supported the observed temperature-dependent metabolic response. The analytical workflow used to monitor microalgal growth was further evaluated using the AGREE metric based on the principles of Green Analytical Chemistry. The estimated AGREE score of approximately 0.76 indicates a moderately green analytical procedure. The results highlight the importance of temperature control in maintaining stable microalgal cultivation and demonstrate the potential of integrating environmental performance assessment into microalgal growth studies.

Keywords: chlorella sp., microalgae cultivation, biomass growth, AGREE green analytical chemistry

ABSTRAK

Perilaku pertumbuhan *Chlorella* sp. dievaluasi pada kondisi suhu yang berbeda untuk meneliti bagaimana variasi termal memengaruhi produksi biomassa mikroalga dan dinamika kultur. Eksperimen budidaya dilakukan pada suhu 28, 35, dan 40 °C di bawah iluminasi kontinu dengan aerasi. Akumulasi biomassa dipantau melalui pengukuran kerapatan optik pada 680 nm, sementara parameter fisikokimia termasuk pH dan total padatan terlarut (TDS) dicatat selama budidaya. Akumulasi biomassa yang stabil diamati pada suhu 28 °C, sedangkan pertumbuhan menjadi kurang konsisten pada suhu 35 °C dan menurun pada suhu 40 °C, menunjukkan peningkatan stres termal pada suhu yang lebih tinggi. Variasi pH dan TDS selama budidaya mendukung respons metabolik yang bergantung pada suhu yang diamati. Alur kerja analitik yang digunakan untuk memantau pertumbuhan mikroalga selanjutnya dievaluasi menggunakan metrik AGREE berdasarkan prinsip-prinsip Kimia Analitik Hijau. Skor AGREE yang diperkirakan sekitar 0,76 menunjukkan prosedur analitik yang cukup ramah lingkungan. Hasil penelitian ini menyoroti pentingnya pengendalian suhu dalam menjaga kestabilan budidaya mikroalga

dan menunjukkan potensi pengintegrasian penilaian kinerja lingkungan ke dalam studi pertumbuhan mikroalga.

Kata kunci: *Chlorella sp.*, budidaya mikroalga, pertumbuhan biomassa, kimia analitik hijau AGREE

INTRODUCTION

Microalgae have attracted considerable attention as a renewable biological resource due to their rapid growth and ability to convert inorganic nutrients into biomass (Fenton, 2012). Their potential applications include biofuel production, wastewater treatment, and the synthesis of valuable biochemical compounds (Hoang et al., 2023). Among various microalgal species, *chlorella sp.* has been widely investigated because of its relatively high biomass productivity and adaptability to different environmental conditions (Daliry et al., 2017). The growth of microalgae is strongly influenced by environmental parameters such as nutrient availability, light intensity, and temperature (Masojídek et al., 2021). Temperature plays a particularly important role because cellular metabolism relies heavily on enzyme-mediated reactions that are sensitive to thermal conditions (Masojídek et al., 2021). Changes in temperature can affect photosynthetic efficiency, nutrient assimilation, and membrane stability, ultimately influencing biomass productivity (Lau et al., 2022).

Microalgal growth typically occurs within a limited thermal window (Vuppaladadiyam et al., 2018). When

temperature conditions are favorable, metabolic reactions proceed efficiently and support continuous biomass accumulation (Schediwy et al., 2019). In contrast, temperatures exceeding the optimal range may disrupt enzymatic activity, impair photosynthetic processes, and reduce overall culture stability (Ras et al., 2013). Understanding the temperature response of microalgal systems is therefore essential for designing stable cultivation processes (Ras et al., 2013). Despite extensive research on microalgal cultivation, the combined evaluation of biomass growth and physicochemical culture dynamics under varying temperature conditions remains relatively limited for laboratory-scale *chlorella* systems (Ahamefule et al., 2025). Many studies focus primarily on biomass productivity without simultaneously examining how temperature influences both biological growth and chemical equilibrium within the culture medium (Chowdury et al., 2020; Gatamaneni et al., 2018). Monitoring parameters such as optical density, pH, total dissolved solids, and inorganic carbon dynamics can provide additional insight into metabolic responses during cultivation (Geltner et al., 2025; Havlik et al., 2022a).

In this study, the growth behavior of *Chlorella sp.* was evaluated under controlled cultivation conditions at different temperature levels. Optical density measurements were used to monitor biomass accumulation, while variations in physicochemical parameters were recorded to provide additional insight into culture dynamics (Havlik et al., 2022b). In this context, the present study distinguishes itself by combining growth analysis with physicochemical monitoring and proposing a carbon transformation pathway to interpret the observed trends, while also incorporating a greenness assessment of the analytical workflow using the AGREE approach (Imam & Abdelrahman, 2023).

MATERIALS AND METHODS

Microalgal Cultivation

Cultures of *Chlorella sp.* were maintained in liquid growth medium under laboratory conditions. The cultures were placed in aerated flasks and exposed to continuous illumination to support photosynthetic activity. The culture medium was prepared using a commercial NPK fertilizer (Growmore, 32:10:10) as the primary nutrient source. 10 g of fertilizer was dissolved in 10 L of distilled water to provide essential macronutrients, particularly nitrogen, phosphorus, and potassium required for microalgal growth. The initial inoculum was added at

approximately 10% (v/v) of the working volume. Temperature conditions were adjusted to 28°C, 35°C, and 40°C to evaluate the influence of thermal variation on microalgal growth. All cultivation experiments were conducted under identical conditions except for temperature. Continuous aeration was supplied to maintain mixing within the culture medium and to ensure adequate gas exchange during cultivation.

Growth Monitoring

Microalgal growth was monitored using optical density measurements at 680 nm using a UV-Vis spectrophotometer. Optical density is commonly used as an indirect indicator of biomass concentration because chlorophyll pigments strongly absorb light within this wavelength range (Griffiths et al., 2011). Measurements were conducted periodically during the cultivation period to observe biomass accumulation trends under different temperature conditions. The specific growth rate was estimated using the equation 1. where x_1 and x_2 represent optical density values measured at time t_1 and t_2 , respectively.

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (1)$$

Physicochemical Analysis

Supporting physicochemical parameters including pH and TDS were

measured throughout the cultivation period. These parameters provide additional information regarding nutrient utilization and metabolic activity occurring within the culture system.

Greenness Assessment using AGREE

The environmental performance of the analytical workflow applied in this study was evaluated using the AGREE metric, which is based on the twelve principles of Green Analytical Chemistry (Imam & Abdelrahman, 2023). This framework provides a semi-quantitative assessment of analytical methods by considering factors such as reagent consumption, energy requirements, waste generation, and operational safety (Imam & Abdelrahman, 2023). The evaluation was conducted by examining the procedures used for monitoring microalgal cultivation, including biomass estimation, physicochemical measurements, and carbon equilibrium analysis. Each analytical step was assessed according to the relevant AGREE criteria, including sample preparation requirements, reagent consumption, waste generation, energy demand, and operator safety.

Biomass growth was monitored using spectrophotometric measurements at 680 nm, which allow direct estimation of microalgal concentration without chemical derivatization or solvent extraction (Berden-Zrimec et al., 2026). Figure 1

showed the schematic representation of the experimental procedure and analytical workflow for evaluating temperature-dependent growth of *Chlorella* sp., including cultivation conditions, measurement parameters, and greenness assessment using the AGREE framework.

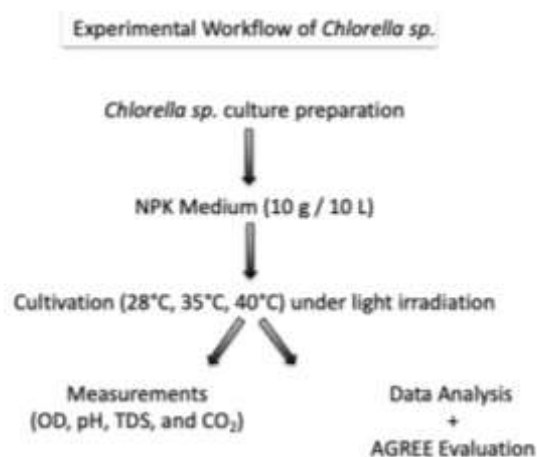


Figure 1. Schematic diagram of experimental procedure and analytical workflow

Physicochemical parameters such as pH and total dissolved solids were measured directly within the culture system using portable instruments. Additional carbon-related measurements were performed using titration-based analysis involving small volumes of acid and indicator reagents. Each of the twelve AGREE criteria was assigned a score ranging from 0 to 1 based on the environmental impact of the analytical procedure. The overall greenness score was calculated as the average value of these individual criteria, providing an overall estimate of the environmental compatibility

of the analytical workflow used in this study.

RESULTS AND DISCUSSION

Temperature Dependent Growth Behavior

The growth profile of *chlorella sp.* exhibited a clear dependence on cultivation temperature in five days. Optical density measurements indicated that cultures maintained at 28°C experienced continuous biomass accumulation throughout the cultivation period. The quantitative growth data are summarized in Table 1, which shows that the culture grown at 28°C reached the highest optical density value (0.22), corresponding to the highest estimated growth rate (0.46 day⁻¹).

Table 1. Optical density and growth rate of *chlorella sp.* under different temperature conditions

Temperature (°C)	Initial OD (680 nm)	Maximum OD (680 nm)	Estimated growth rate, μ (day ⁻¹)
28	0.02	0.22	0.46
35	0.02	0.18	0.39
40	0.02	0.11	0.27

When the cultivation temperature was increased to 35°C, biomass accumulation remained detectable but the maximum optical density decreased to

approximately 0.18. The corresponding growth rate was slightly lower (0.39 day⁻¹), indicating that the culture approached the upper boundary of its optimal temperature range. A markedly different trend was observed at 40°C. The maximum optical density reached only about 0.11 with a considerably lower growth rate (0.27 day⁻¹). This decline suggests that prolonged exposure to elevated temperature conditions reduces metabolic stability and limits biomass accumulation (Walker et al., 2018).

Effect of Temperature on *Chlorella sp.* Growth

The growth profile of *chlorella sp.* showed clear temperature dependence, as shown in Figure 2. At 28°C, optical density increased steadily, indicating favorable physiological conditions. Growth at 35°C was still observable but less consistent, suggesting mild thermal stress. At 40°C, a decline in optical density occurred after initial growth, indicating reduced cellular stability.

Microalgal metabolism is sensitive to temperature because enzymatic activity and photosynthetic efficiency operate within a limited range (Kumari et al., 2022). Unlike chemical hydrolysis, which benefits from increased thermal input, biological systems exhibit optimal performance only within specific thermal windows (Ometto et al., 2014). The present results confirm that

28°C represents a suitable condition for stable biomass accumulation in this system.

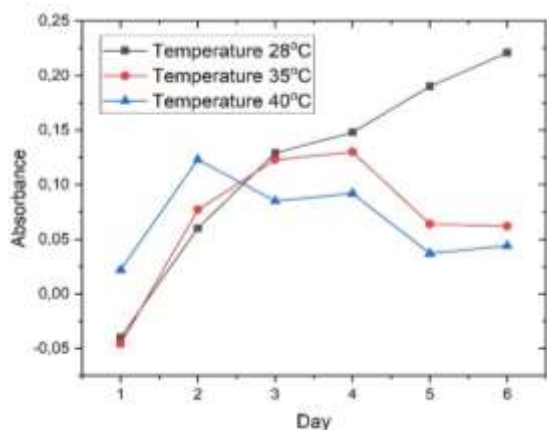


Figure 2. The effect of temperature on the growth of *chlorella* sp.

Temperature plays a central role in regulating microalgal metabolism because many intracellular processes are governed by enzyme-mediated reactions (Elisabeth et al., 2021). Under moderate temperature conditions, enzymatic reactions proceed efficiently, allowing photosynthesis, carbon fixation, and nutrient assimilation to occur in a balanced manner. This balanced metabolic state is reflected in the stable biomass accumulation observed at 28°C. When temperature increases beyond the optimal range, several physiological limitations may arise. Elevated temperature can destabilize photosynthetic complexes, disrupt electron transport processes, and alter membrane fluidity. These changes may reduce metabolic efficiency and limit biomass production. The decline in optical density observed at 40°C suggests that *chlorella* cells experienced thermal stress

under these conditions. Prolonged exposure to elevated temperature may impair cellular metabolism and prevent sustained biomass accumulation.

Variation of Physicochemical Culture Parameters

Changes in physicochemical parameters during cultivation provided additional insight into the metabolic activity of the microalgal culture. The variation of pH and total dissolved solids during cultivation is presented in Figure 3. The pH of the culture medium exhibited gradual variation throughout the cultivation period. These changes are commonly associated with photosynthetic activity, during which microalgae consume dissolved carbon dioxide. The consumption of carbon dioxide reduces carbonic acid concentration in the medium and can result in an increase in pH.

The relatively stable pH observed during the 7-day cultivation period can be explained by the dynamic balance between photosynthetic CO₂ uptake and respiratory CO₂ release within the culture system. During photosynthesis, microalgae consume dissolved CO₂, which tends to increase pH, while respiration and carbon equilibrium reactions contribute to CO₂ release, counteracting this effect. As a result, the overall pH remains relatively stable despite ongoing metabolic activity. Similar behavior has been reported in

microalgal systems, where pH variations are often buffered by the equilibrium between dissolved CO₂, bicarbonate, and carbonate species (Popa et al., 2025).

In contrast, the observed variation in total dissolved solids (TDS) reflects changes in dissolved nutrients and metabolites during cultivation. Microalgae actively assimilate inorganic nutrients such as nitrogen and phosphorus from the medium for biomass formation, which can reduce dissolved solids over time. At the same time, the release of metabolic by-products or cellular components may contribute to fluctuations in TDS values. Previous studies have shown that microalgae play an important role in nutrient uptake and transformation, leading to measurable changes in dissolved components of the culture medium (Drazdienė et al., 2024). Additionally, elevated TDS levels may influence osmotic conditions in the culture system without necessarily causing significant pH variation (Singh et al., 2025).

These results indicate that pH alone may not fully capture the dynamic changes occurring in the culture medium, whereas TDS provides complementary information related to nutrient utilization and metabolic activity during microalgal growth.

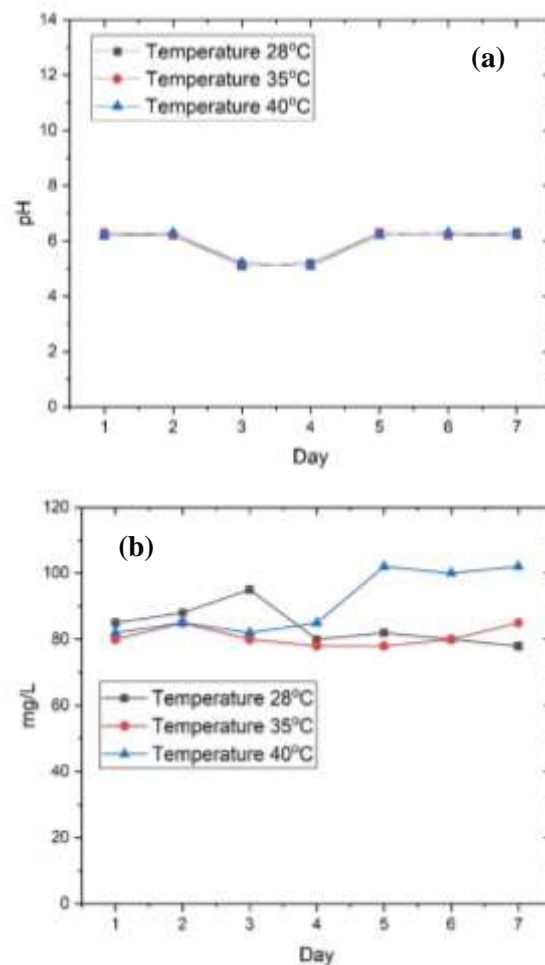


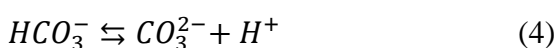
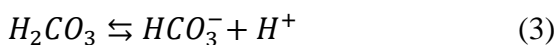
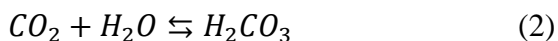
Figure 3. The experiments parameters variation of (a) pH and (b) TDS

Total dissolved solids also showed variation during cultivation. Changes in TDS may reflect nutrient uptake by microalgal cells as well as the transformation of dissolved compounds within the medium. Although the magnitude of change was moderate, the observed trend indicates that dissolved components of the culture medium were gradually modified during biomass growth.

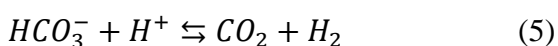
Proposed Carbon Assimilation and Transformation Mechanism

The growth of *chlorella* sp. during cultivation is closely associated with the transformation of inorganic carbon species present in the culture medium. Dissolved carbon dioxide participates in a series of equilibrium reactions that regulate carbon availability for microalgal metabolism. These reactions are coupled with photosynthetic carbon fixation processes that ultimately lead to biomass formation.

In aqueous systems, dissolved carbon dioxide first interacts with water to form carbonic acid to form carbonate ions according to the following equilibrium in reaction (2 – 4).

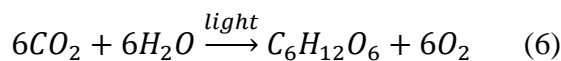


These equilibria regulate the distribution of inorganic carbon species within the culture medium. Microalgal cells can utilize both dissolved carbon dioxide and bicarbonate as carbon sources. Bicarbonate ions can be converted into carbon dioxide through the reaction (5).



The resulting carbon dioxide is then assimilated by *chlorella* sp. cells through photosynthetic carbon fixation reactions. In

the presence of light, carbon dioxide and water are converted into carbohydrates according to the simplified photosynthetic reaction (6).



The produced carbohydrates are subsequently utilized in various biosynthetic pathways for the formation of cellular components such as proteins, lipids, and structural carbohydrates. This process can be represented in simplified form as reaction (7).



As photosynthetic carbon fixation proceeds, dissolved carbon dioxide is gradually consumed from the culture medium. This consumption shifts the carbonate equilibrium and can reduce the concentration of carbonic acid. As a consequence, the pH of the culture medium may increase slightly during active microalgal growth. The coupling between inorganic carbon equilibria and photosynthetic assimilation therefore explains the simultaneous changes observed in biomass concentration, pH, and dissolved solids during cultivation (Peng et al., 2025). This proposed reaction scheme illustrates how biological growth and chemical equilibria interact within the microalgal culture system.

Greenness Assessment

The environmental profile of the analytical workflow used in this study was evaluated using the AGREE metric based on the twelve principles of Green Analytical Chemistry. The evaluation focused on the experimental procedures applied during biomass monitoring and physicochemical analysis of the culture medium, as shown in Figure 4.

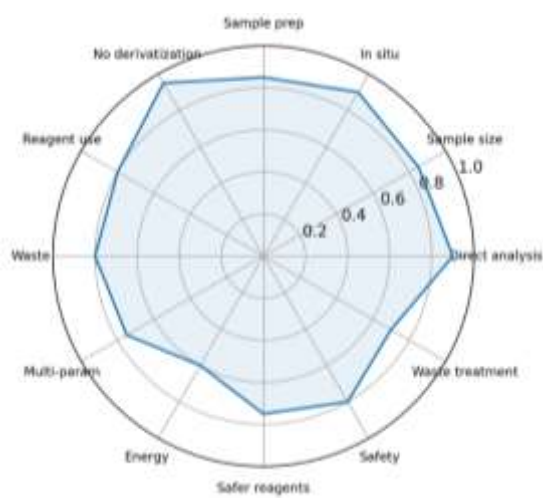


Figure 4. AGREE radar chart representing the greenness profile of the analytical workflow

The overall AGREE score of the analytical procedure was estimated at approximately 0.76, indicating a moderately green analytical workflow (Semysim et al., 2025). The relatively favorable score is mainly attributed to the use of direct spectrophotometric measurements for biomass estimation. Optical density measurements at 680 nm allow rapid monitoring of microalgal growth without requiring solvent extraction

or destructive sample preparation, thereby minimizing reagent consumption and chemical waste generation. Additional parameters such as pH and total dissolved solids were measured directly in the culture medium using portable instruments. These in situ measurements further reduce the need for complex sample processing and contribute to the environmental compatibility of the analytical procedure.

The analytical workflow demonstrates a reasonable balance between analytical reliability and environmental sustainability. The integration of direct spectrophotometric monitoring with minimal reagent use supports the implementation of greener analytical practices in microalgal cultivation studies.

CONCLUSIONS

The growth behavior of *chlorella sp.* was strongly influenced by cultivation temperature. Stable biomass accumulation occurred at 28°C, whereas higher temperatures reduced growth stability. Cultures maintained at 35°C showed moderate growth, while cultivation at 40°C induced thermal stress that limited biomass production. Variations in physicochemical parameters during cultivation further supported the observed temperature-dependent metabolic response. Changes in pH and total dissolved solids reflected

metabolic activity and nutrient utilization within the culture system.

The greenness evaluation using the AGREE framework indicated that the analytical workflow applied in this study can be categorized as moderately green, largely due to the use of direct spectrophotometric measurements and minimal reagent consumption. These findings highlight the importance of temperature control in maintaining stable microalgal cultivation systems and demonstrate the value of integrating environmental assessment into experimental workflows for biomass research.

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